

**CLAIMS:**

1. A method for determining the morphology and motility of a population of cells in vitro including the steps:

5 (a)(i) capturing a first frame of image data of said population and identifying a part or parts of the image data corresponding to a cell or cells of interest

10 (a)(ii) capturing a second frame of image data of said population and identifying a part or parts of the image data corresponding to a cell or cells of interest

(b)(i) determining the morphology of the cell or cells of interest from the first and/or second frame

15 (c)(i) determining the relative displacement, in the second frame compared to the first frame, of the cell or cells of interest.

2. A method according to claim 1 wherein the first and  
20 second frames are adjacent frames in a series of more than two frames of image data captured of said population, the method further including, for each frame of said series, the steps:

25 (a)(iii) identifying a part or parts of said image data corresponding to the cell or cells of interest

(c)(ii) determining the relative displacement, in said frame compared to the previous frame in said series, of the cell or cells of interest.

3. A method according to claim 2, the method further including the step:

5 (b) (ii) determining the morphology of said cell or cells identified in step (a) (iii).

4. A method according to any one of claims 1 to 3 including the step of determining a value for the motility of the cell or cells of interest, based on the  
10 relative displacement of the cell or cells of interest.

5. A method according to any one of claims 1 to 4 including the step of determining the amount or relative amount of the population of cells having a motility at or  
15 above a threshold motility value.

6. A method according to any one of claims 1 to 5 including the step of classifying the cell or cells of step (b) (i) or step (b) (ii) as morphologically normal or  
20 morphologically abnormal.

7. A method according to claim 6 including the step of determining the amount or relative amount of the population of cells being morphologically normal.  
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8. A method according to any one of claims 4 to 7 including the step of determining the amount or relative amount of the population of cells:

being morphologically normal, and  
having a motility at or above a threshold motility  
value.

5 9. A method according to any one of claims 1 to 8 for  
carrying out a first determination of morphology and  
motility on a first area of a sample of cells, the method  
including the step of carrying out a second and,  
optionally, further, determinations of morphology and  
10 motility on a second and, optionally, further, areas of  
the sample.

10. A method of processing image data captured from a  
population of cells in vitro in order to determine the  
15 morphology and motility of the cells, the image data  
including a first frame of image data of said population  
and a second frame of image data of said population, the  
method including the steps:

(i) determining the morphology of the cell or cells  
20 of interest from the first and/or second frame; and  
(ii) determining the relative displacement, in the  
second frame compared to the first frame, of the  
cell or cells of interest.

25 11. A method according to any one of claims 1 to 10  
wherein the first frame of image data is processed to  
identify illumination intensity distributions of interest  
having one of a plurality of characteristic profiles.

12. A method according to claim 11 wherein one of the characteristic profiles is a first characteristic profile having a centre point of a relatively high intensity  
5 surrounded by a substantially symmetrical gradual reduction in intensity.

13. A method according to claim 11 or claim 12 wherein the parts of the image data corresponding to the  
10 illumination intensity distributions of interest are further processed to identify cell perimeter features surrounding one or more of said illumination intensity distributions of interest.

14. A method according to any one of claims 11 to 13 wherein an object of interest is identified, the method further including the step of determining one or more dimensions or relative dimensions of an object.

15. A method according to claim 14 wherein said dimensions or relative dimensions are compared to one or more predetermined ranges of corresponding dimensions or relative dimensions.

16. A method according to claim 14 or claim 15 further including the step of determining whether said object is a cell to be tracked or not and:

if said object is a cell to be tracked, assigning a tracking identity to it; or  
if said object is not a cell to be tracked, assigning a residual object identity to it.

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17. A method according to any one of claims 11 to 16 further including the step of determining a characteristic morphological value for said cell to be tracked.

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18. A method according to any one of claims 11 to 17 repeated in order to identify all cells to be tracked and all objects not to be tracked in a frame of image data.

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19. A method according to any one of claims 11 to 18 repeated for the second and/or subsequent frames.

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20. A method according to claim 19 further including tracking the cells by identifying said cells and their locations in the second and/or subsequent frames of image data.

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21. A method according to claim 20 wherein when the tracks of two cells of interest intersect, the cells and their tracks are identified before and after the intersection by their characteristic morphologies.

22. A method according to claim 20 or claim 21 wherein the a cell of interest is identified in one frame and not identified in the next frame, the cell being identified in a subsequent frame, further including calculating tracking data to connect the track of the cell through the frames.

23. A method according to any one of claims 18 to 22 further including the step of determining a motility characteristic for a tracked cell.

24. A method according to claim 23 further including determining an overall figure of merit for the sample indicative of the number or proportion of morphologically normal cells with normal motility.

25. A method according to any one of claims 1 to 24 wherein image capture is performed using digital imaging means providing a frame resolution or an effective frame resolution of at least  $0.5 \times 10^6$  pixels.

26. A method according to any one of claims 1 to 25 wherein the rate of image capture for a series of frames is at least 20 Hz.

27. A method according to any one of claims 1 to 26 wherein the cell or cells of interest are spermatozoa.

28. A method according to claim 27 wherein the cell or cells of interest are human spermatozoa.

29. A method according to any one of claims 1 to 28 further including the step of diagnosis based on the determination of the morphology and motility of the population of cells in vitro.

30. A method according to claim 29 wherein the step of diagnosis is based on a value of the amount or relative amount of cells categorised as morphologically normal and having a motility at or above a threshold motility value.

31. Apparatus for determining the morphology and motility of a population of cells in vitro, the apparatus including:

imaging means for capturing first and second frames of image data of said population and identifying a part or parts of the image data corresponding to a cell or cells of interest;

computation means for determining the morphology of the cell or cells of interest from the first and/or second frame and for determining the relative displacement, in the second frame compared to the first frame, of the cell or cells of interest.

32. Apparatus according to claim 31 wherein the imaging means is digital imaging means providing a frame

resolution or an effective frame resolution of at least  
0.5 x 10<sup>6</sup> pixels.

33. Apparatus according to claim 31 or claim 32 wherein  
5 the imaging means is arranged to capture a series of  
frames at a rate of at least 20 Hz.

34. Apparatus according to any one of claims 31 to 33  
wherein the imaging means includes phase contrast optics.  
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35. Apparatus according to any one of claims 31 to 34  
for carrying out the method of any one of claims 1 to 35.

36. A computer system operatively configured to carry  
15 out the method according to any one of claims 1 to 30.

37. Computer programming code for operatively  
configuring a computer system to carry out the method  
according to any one of claims 1 to 30.  
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38. A data carrier having recorded on it computer  
programming code according to claim 37.